

Enzyme-passage free culture of mouse embryonic stem cells on thermo-responsive polymer surfaces

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Supporting Information

Table S1: PCR primer sequences and RT-PCR details

Gene	Cycle No.	Primer-Probe Sequence 5'-3'	Product Size (bp)	Annealing temp (°C)
GAPDH	30	F: TGAGGCCGGTGCTGAGTATGTCTG R: CCACAGTCTTCTGGGTGGCAGTG	302	60
Oct-4	30	F: AGCACGAGTGGAAAGCAACT R: AGATGGTGGTCTGGCTGAAC	248	60
GATA4	30	F: CTGGAAGACACCCCAATCTC R: GTAGTGTCCCGTCCCATCTC	130	55
Brachyury	30	F: GCTGTTGGGTAGGGAGTCAA R: CCCCGTTCACATATTTCCAG	380	60
Nestin	30	F: AGGCGCTGGAACAGAGATT R: TTCCAGGATCTGAGCGATCT	150	55

Table S2: Elemental compositions for plasma polymerised allyl alcohol (ppAAI) surfaces, ppAAI 2-bromo-isobutyrate surfaces and Poly (MEO₂MA-co-OEGMA) graft surfaces.

Assignments	Quantification (Atomic %)		
	ppAAI	ppAAI-2-bromo-isobutyrate	poly(MEO ₂ MA-co-OEGMA)
C1s	85.2±0.1	85.05± 1.5	74.8±1.2
O1s	14.8±0.3	12.3±1.11	18.44±0.8
Si2p	-	1.2±0.3	-
NaKLL	-	0.5± 0.05	0.7±0.01
Ca2p	-	-	1.6±0.2
Zn2p	-	-	0.24±0.3
Br3d	-	0.7± 0.08	0.21±0.03
Mg1s	-	-	4.01 ± 0.04

Table S3: XPS C1s core level curve fit results for Poly (MEO₂MA-co-OEGMA) graft surfaces.

Assignments	Binding energy/eV	Quantification (atomic %)	Notes
C -C/C-H	285.021	77.4±0.7	Polymethacrylate backbone
O-C(=O)-<u>C</u>-CH₃-secondary shift	285.721	2.9±0.09	End group of poly(OEGMA)
C-O	286.659	13.4±0.46	
O-C-O/C=O	287.835	3.4±0.1	
<u>C</u>(=O)OCH₂	289.221	2.9±0.08	

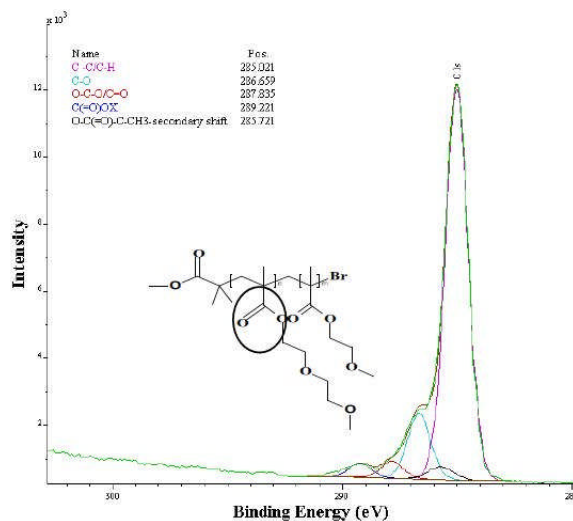


Figure S1: C1s core level spectra recorded after growth from the surface of poly(MEO₂MA-co-OEGMA) brushes via ATRP. Component peaks from the poly(methacrylate) backbone include C-C/C-H and C(=O)OX and the end group of poly(OEGMA).

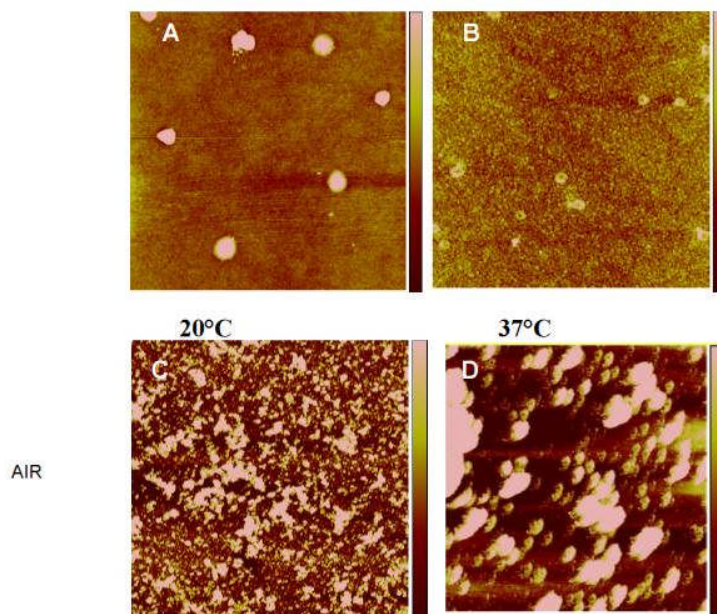


Figure S2: (A) Plasma polymerised allyl alcohol (ppAAI). (B) bromo-isobutyryl bromide imaged using AFM in air. Representative AFM images of poly (MEO₂MA-co-OEGMA) grafted surface below (20°C) (C) and above (37°C) (D) the LCST in air (scan size: 5*5 μm², z-range: 10 nm).

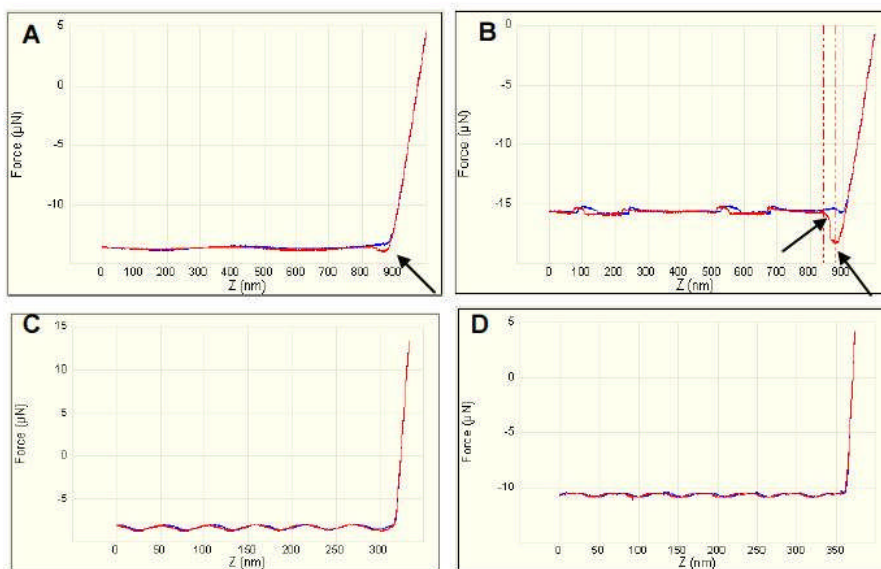


Figure S3: Representative force distance curves obtained for ozone cleaned Si₃N₄ AFM tip at poly (MEO₂MA-co-OEGMA) surfaces. At 20°C (A) low adhesion between tip and surface indicated by small pull-off trace (arrowed) in contrast to the higher pull-off force observed at 37°C. (B) PEOGMA control surfaces showed no adhesion between the AFM tip and the polymer layer as it has an LCST of 90°C, below (C) and above 37°C (D).

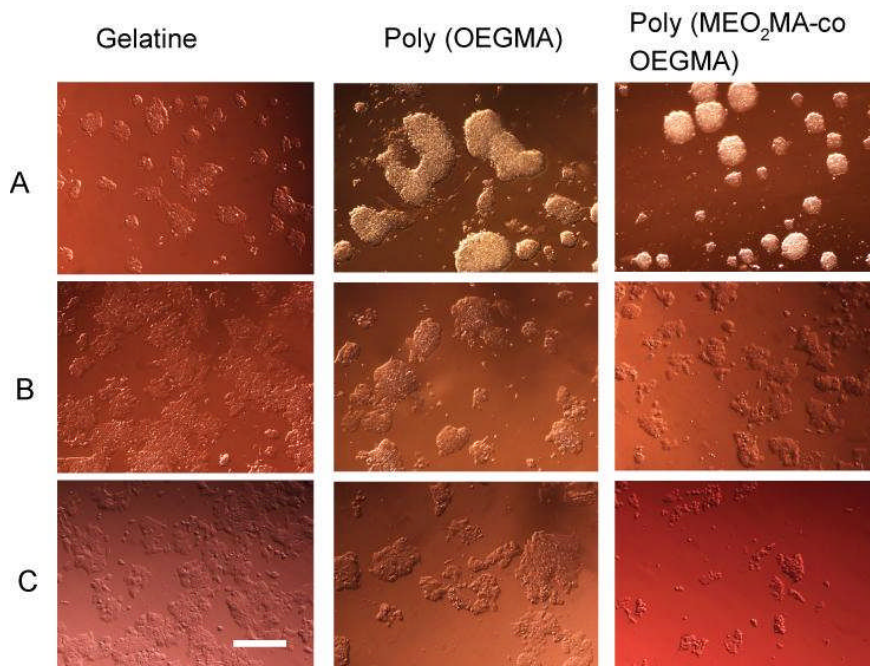


Figure S4: Feeder free mES cells attachment studies to Gelatine, poly(OEGMA) and poly(MEO₂MA-co-OEGMA). (A) Low attachment of mESCs to synthetic polymer surfaces and formation of embryoid bodies compared to gelatine (positive control). (B) mES cell attachment to poly(OEGMA) and poly(MEO₂MA-co-OEGMA) after treatment with fibronectin compared to gelatine. (C) Low detachment of cells from gelatine, partial retention of cells on poly(OEGMA) but reduced cell attachment to poly(MEO₂MA-co-OEGMA). Scale bar = 100µm.

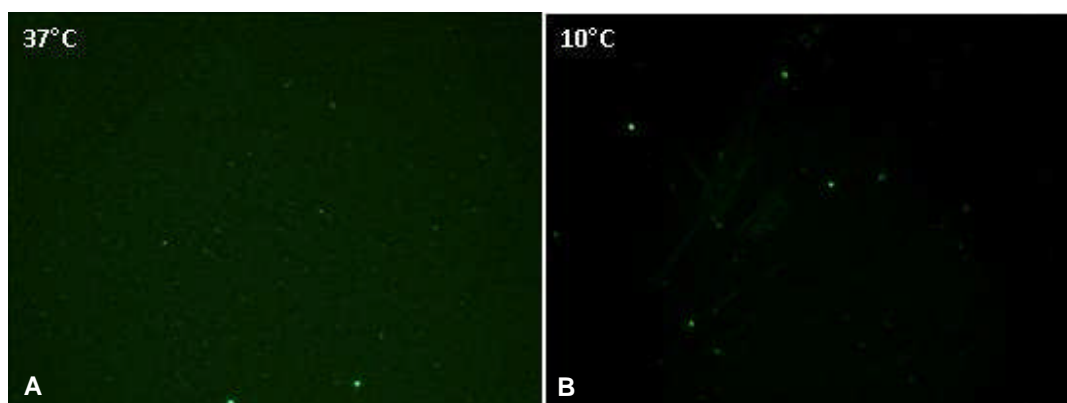


Figure S5: Representative fluorescence images of surfaces immuno-stained with fluorescent antibodies to fibronectin after incubation of fibronectin with poly(MEO₂MA-co-OEGMA) at 37°C overnight (A) and after detachment of cells at 10°C (B).

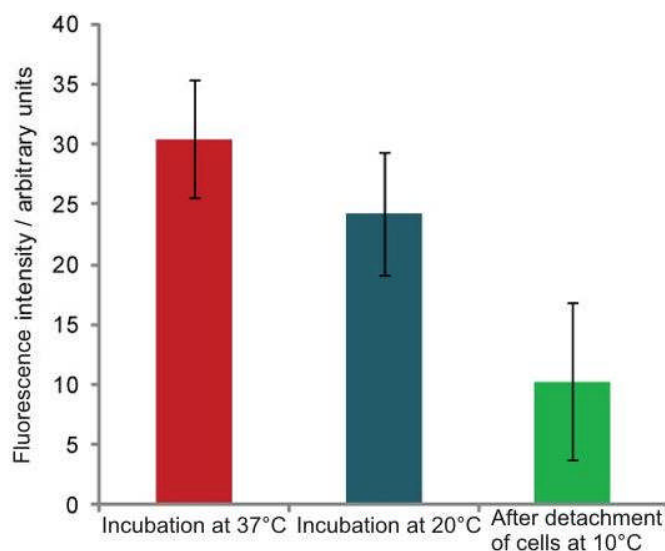


Figure S6: Averaged fluorescence intensities of poly(MEO₂MA-co-OEGMA) surfaces following incubation with fibronectin, washing and then immunostaining with a FITC-anti fibronectin antibody. Error bars represent SD and n=3

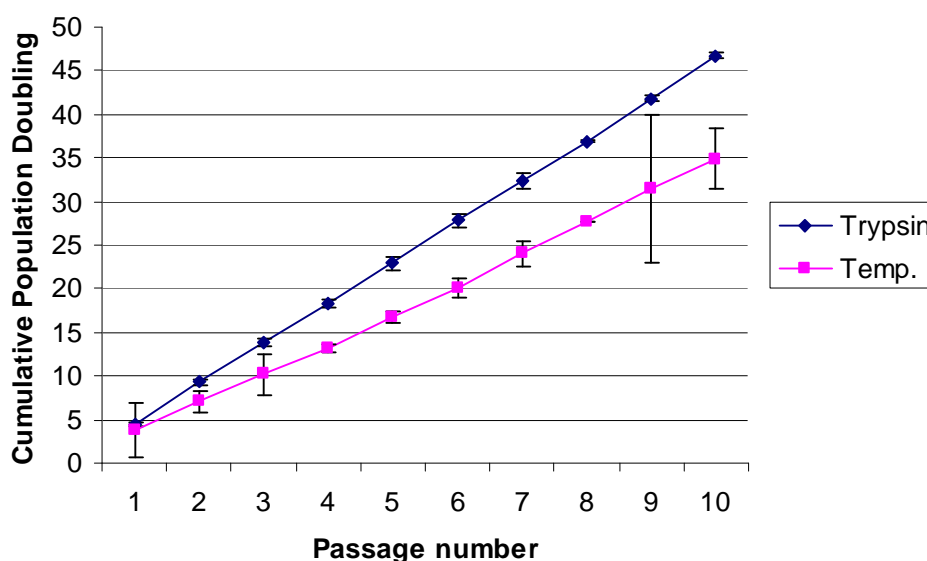


Figure S7: Growth curve of mESCs which were serially passaged using trypsin (◆) and temperature (◻) from gelatine coated TCPS (control) or the poly (MEO₂MA-co-OEGMA)/fibronectin thermoresponsive polymer surface respectively over 10 passage cycles (error bars indicate SD; n = 9, three individual samples in a total of three separate experiments)